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Histocompatibility Genes of Mice

III. *H-1* AND *H-4*, TWO HISTOCOMPATIBILITY LOCI IN THE FIRST LINKAGE GROUP

G. D. SNELL AND L. C. STEVENS

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine U.S.A.

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Summary. A new histocompatibility locus, *H-4*, in the first linkage group of the mouse, has been identified. The gene-order in this linkage group is *H-1*, *c*, *p*, with *H-4* very close to *p* but its position relative to *p* undetermined. There is 8.5 ± 2.1 per cent crossing over between *H-1* and *c* in the heterozygous female, 5.4 ± 3.7 per cent in the heterozygous male. At least 4 alleles at the *H-1* locus have been identified. The distribution of *H-1* alleles in various inbred and co-isogenic resistant stocks is shown in Table 11. The mean survival of skin grafts made between various co-isogenic strain pairs, differing at *H-1*, ranges from 32 to 91 days, with a minimum survival of individual grafts of 20 days and a maximum of 124 days. Ovarian grafts between the same strain pairs show much longer survival. The significance of these results for estimates of gene numbers is discussed; the higher published estimates are favoured.

INTRODUCTION

A previous study (Snell, 1958b) has shown that a histocompatibility locus, defined by the co-isogenic strains C3H and C3H.K and designated *H-1*, is linked with albinism (*c*) and manifests about 8 per cent recombination with this locus. This report gives further information as to the linkage relations of *H-1*, describes a new locus, *H-4*, in the same (first) linkage group, and presents evidence as to multiple alleles at *H-1*. The results of normal tissue transplants between lines differing at *H-1* are also given.

MATERIALS AND METHODS

MICE. The strains of mice used, and the substrain thereof where this is significant, are listed in Table 1. Three of these strains, C3H, C57BL/10, and 129, are standard inbred lines. The others are co-isogenic (or congenic) lines produced by methods previously described (Snell, 1958b). These methods involve a series of crosses which make the co-isogenic strain nearly identical with the standard strain, except, however, for a single histocompatibility difference, and for a segment of chromosome of undetermined but usually short length introduced with the histocompatibility difference. Borne in this chromosome segment may also be a gene determining a coat colour difference. By way of example, strain C3H.K is differentiated from strain C3H, with which it is approximately co-isogenic, by the presence of a chromosome segment, derived from strain K, and carrying the genes $H\text{-}1^b$ and *c* (albinism). C3H is $H\text{-}1^a$ and *C*. The co-isogenic strains

doubtless differ also from their partners by other minor differences, which may or may not include histocompatibility differences. The number of such differences is determined partly by chance, but more importantly by the number of generations through which the crosses used to produce the strains are pursued. We have usually regarded fourteen generations (abbreviated G14) as the minimum necessary to produce adequate co-isogenicity; many lines are carried to G18. The last column of Table 1 summarizes available information from this paper and from prior publications about the histocompatibility genotypes of the strains. Most of the new information concerns strains B10.129(5M), B10.129(21M), and B10.129(20M) which were produced by an initial cross between C57BL/10 and 129. The histocompatibility genotypes of all other lines listed in the table have already been analysed to a greater or less degree (Snell, 1958b).

TUMOURS. The tumours used were C3H leukaemia E9514, C57BL/6 leukaemia C1498 and C57BL/10 leukaemia S913. All tumours were routinely carried by subcutaneous transplant.

TABLE 1

LIST OF STRAINS

Strains	*Synonyms*	*Genotype**
C3H/HeDiSn	C3H	*H-1^a/H-1^a*
C3H.K	C3H-*H-1^{b}c*	*H-1^{b}c/H-1^{b}c*
C57BL/10ScSn	C57BL/10 or B10	*H-1^{c}H-4^a/H-1^{c}H-4^a H-2^b/H-2^b H-3^{a}a/H-3^{a}a*
C57BL/6Jax	C57BL/6	Slight difference from C57BL/10 not genetically analysed
B10.BY	C57BL/10-*H-1^d*	*H-1^{d}H-4^a/H-1^{d}H-4^a*
B10.129(5M)	5M	*H-1^{e}cH-4^a/H-1^{e}cH-4^a*†
B10.129(21M)	21M	*H-1^{c}pH-4^b/H-1^{c}pH-4^b*
B10.129(20M)	20M	*H-1^{c}cpH-4^b/H-1^{c}cpH-4^b*
B10.D2	C57BL/10-*H-2^d*	*H-2^d/H-2^d*
B10.LP	C57BL/10-*H-3^{b}A^w*	*H-3^{b}A^w/H-3^{b}A^w*
129/Sn	129	*H-1^{e}c^{c}h^{h}pH-4^b/H-1^{e}c^{c}h^{h}pH-4^b*† *H-2^b/H-2^b*

* In the case of coat colour genes, only mutant genes are listed. In the case of histocompatibility genes, usually only differences of the isogenic resistant strain from its co-isogenic, standard-strain partner are listed.

† The *H-1* allele in strains 5M and 129, listed as *H-1^e*, has not been tested against the allele in C3H.K and may, therefore, be *H-1^b*.

For experimental use, a cell suspension was prepared in the cytosieve (Snell, 1953), counted, diluted to the required concentration, and implanted subcutaneously. Tumours were palpated weekly, or, when survival time was important, at less than weekly intervals, and the approximate size of growth recorded. Animals were regarded as positive or susceptible only where they succumbed to the tumour.

IMMUNIZATION. In the study of 'weak', non-*H-2* differences, it is routine practice to immunize with donor thymus before tumour transplantation (Snell, 1958a). A suspension of thymus was prepared in the cytosieve and injected intra-abdominally. In early experiments, a single injection of about 10 million cells per mouse was made, with tumour inoculated about 11 days later. In later experiments, mice received three injections at the rate, successively, of one donor per thirty hosts, one donor per twenty hosts and one donor per ten hosts. Injections were 1 week apart; tumour was given 1 week after the last injection. Tests (unpublished) have proved the value of the multiple immunizing injections.

SKIN GRAFTS. A 5 mm. square of skin was removed from each side of the ears of donor

animals. Most of the cartilage was scraped from the grafts which were placed upon the panniculus carnosus in the mid dorsal region of the hosts. The area operated on was covered with petroleum jelly-impregnated gauze and the animals were wrapped with self-sealing latex bandage (Bryant and Bernard, 1955). Bandages were left on for 7 days, and grafts were observed with a stereoscopic microscope.

OVARIAN GRAFTS. Ovarian grafts were performed as previously described (Stevens, 1957). Females with ovarian grafts were mated with males of genotypes which made it possible to distinguish positively, by coat colour, offspring of graft from offspring of host origin. The graft survival time was indicated by the reproductive activity of the transplanted ovarian tissue — that period from the date of transplantation to the birth date of the last litter of graft origin.

RESULTS

Table 2 summarizes the results of various control implants of E9514 and S913, and serves to show the capacity of such implants to distinguish between susceptible and

TABLE 2

RESULTS OF CONTROL ISOGRAFTS AND HOMOGRAFTS

Donor strain	*Tumour*	*Recipient strain*	*Tumour dose*	*Fraction dying*	
				*Immunized**	*Unimmunized*
C3H	E9514	C3H	1000×5^3	40/40	
		,,	1000×5^2	10/10	
		C3H.K	1000×5^4	1/20	25/25
		,,	1000×5^3	0/26	
C57BL/10	S913	C57BL/10	1000×5^3	47/47	
		,,	1000×5^4	50/50	
		C57BL/6	,,	0/10	10/10
		B10.BY	,,	0/40	1/23
		B10.129(5M)	,,	0/16	8/28
		B10.129(21M)	,,	0/19	9/10

* Immunized with three injections of donor-strain thymus.

resistant genotypes. All mice receiving isografts succumbed, and, with one exception in 131 animals, all mice receiving homografts after immunization survived. The way in which S913 distinguished between sublines 6 and 10 of strain C57BL indicates the sensitivity of the test. Homografts in un-immunized mice caused deaths with a frequency ranging, according to the tumour and the host strain, from 1 in 23 to 25 in 25. It should be noted that these controls were run as parts of experiments to which they were appropriate; only the control totals are shown here. It should also be noted that tumours E9514 and S913 have been selected for genetic studies after many trials; most tumours do not give equal discrimination of genotype.

While E9514 and S913 give clear results in pure strain mice, a problem has been encountered in the use of S913 in some heterozygous genotypes. We have already reported (Snell, 1958b) that this tumour does not grow as well in 'susceptible' hybrids as in the susceptible homozygote. We have subsequently found the same effect in other X-ray induced tumours native to strain C57BL/10 and C57BL/10 co-isogenic sublines, and have

studied it in additional tests (unpublished). This 'hybrid effect' has the following characteristics. (1) The apparent 'resistance' of the hybrid increases in proportion to the histocompatibility disparity of the parents; it is weak or non-existent in the case of a single non-*H-2* difference, moderately present (for some tumours) in the case of multiple non-*H-2* differences, and strong only with an *H-2* difference. (2) Survival of a hybrid after grafting does not leave a residue of immunity; the proportion of deaths among regrafted survivors is the same as among animals first grafted. (3) The effect is strongly dose-susceptible; deaths increase as the dose is increased.

Without entering into reasons here, we may state that we regard this 'hybrid effect' as due to an abortive graft-*v*-host reaction. It can be countered in genetic studies by, (1) using a large tumour dose, and (2) regrafting survivors. Fortunately in the present study it was rarely a factor since heterozygosis at *H-2* occurred in only a few of the tests.

We now turn to a consideration of results with individual co-isogenic strains.

Co-isogenic strain B10.BY has been reported to carry *H-2* allele *H-2*^{b} (Snell, 1958b).

TABLE 3

RESULTS OF CROSS ♀(C57BL/10 × C3H-*H-1*^{b}*c*) × ♂B10.BY, SHOWING LINKAGE OF RESISTANCE WITH *c* AND ABSENCE OF LINKAGE WITH *A**

Type of cross	*Susceptible*		*Resistant*		*Total*
	CC	*Cc*	*CC*	*Cc*	
BC coupling	14	8	8	27	57
	Aa	*aa*	*Aa*	*aa*	
BC repulsion	15	11	18	19	63

* Of sixty-three backcross mice, fifty-seven were tested genetically for *c* and provide the information on linkage with this gene. Mice were not immunized. Males were inoculated with C57BL/6 tumour C1498, females with C57BL/10 tumour S913. Of twenty-six males, twenty-four ultimately succumbed; results for males are therefore tabulated on the basis of survivors at 4 weeks. There was an excess of negative females, probably due to the 'hybrid effect' which occurs when mice heterozygous for *H-2* are inoculated with S913.

Since strain C57BL/10 with which it is co-isogenic is also H-2^b, the difference between these strains cannot be at the *H-2* locus. A test was therefore set up to see if the locus determining resistance is linked with albinism (*c*), with which *H-1* is associated, or with agouti (*A*) with which *H-3* is associated. The results are shown in Table 3. This test was performed before the value of immunization was appreciated and before we had settled on S913 as our test tumour, and there was undoubtedly a considerable mortality of genetically resistant animals. It was also probably complicated by the 'hybrid effect', which can cause susceptible animals to survive. Despite these complicating factors, it is clear that the histocompatibility difference between C57BL/10 and B10.BY shows linkage with *c*, though the recombination value (28 per cent) is probably higher than the actual cross-over per cent. These results establish a presumption, but do not prove, that B10.BY is an *H-1* line.

Lines B10.129(5M), B10.129(20M) and B10.129(21M) were, as already noted, derived

from an initial cross between C57BL/10 and 129. Since C57BL/10 and 129 are both *H-2*^{b}, we may assume that any difference introduced from the latter on the background of the former is not an *H-2* difference. Further information as to the histocompatibility genotype of these lines can be directly derived from linkage data obtained in the course of their production. Line 129 carries the genes albinism (*c*) and pink-eye (*p*), which are in the first linkage group about 16 crossover units apart. Lines 5M, 20M, and 21M were selected for the presence of these marker genes, 5M for *c* (*p* was still present in some animals at G14 but eliminated by genetic test before G16), 20M for *p* and *c*, and 21M for *p*. Because of the known linkage of *H-1* with *c*, it was assumed that resistance in these lines would segregate in association with *c* and probably also with *p*. Table 4 shows that this was the case. (Data

TABLE 4

SEGRATION OF ALBINISM (*c*) AND RESISTANCE IN LINE B10.129(5M) AND OF PINK-EYE (*p*) AND RESISTANCE IN LINE B10.129(21M). ALL CROSSES F_2 COUPLING

Line	Genera-ation	Number of immunizing injections	Results				Total
			Susceptible		Resistant		
			C	*c*	*C*	*c*	
5M*	14	1	58	2	4(3†)	18	82
	18	3	38	5(1‡)	3	16	62
			P	*p*	*P*	*p*	
21M	14	1 or 2	47	0	4(2§)	17	68
	14	3	16	1	7(5§)	4	28
	16	3	37	0	2	17	56
	18	3	14	1	0	3	18

* Some pairs in G14 were heterozygous for *p* as well as for *c*. One *pp* mouse is omitted from totals.

† Three of four phenotypically resistant *C* mice were progeny-tested and proven genetically resistant. They are therefore classed as crossovers. The fourth mouse is presumed to be a crossover also.

‡ Late death; treated as genetically resistant in calculating crossover per cent.

§ Tested genetically; proven susceptible and hence not crossovers. The survival of these mice presumably indicates that line 21M in generation 14 was segregating for one or more histocompatibility loci in addition to the one linked with *p*.

for 20M are not given, since complicated by the segregation of both *c* and *p*, but linkage of resistance with these genes was equally evident in this line.)

The crossover per cent for 5M in G14 (calculated by the method of Finney, 1949) was 7.8 ± 3.2, and for 5M in G18, 10.9 ± 4.0. These are comparable with published values for *H-1* and *c* (Snell, 1958b) of 7.1 in the heterozygous female and 9.1 in the heterozygous male. The similarity of these values establishes a strong presumption that the gene for resistance in line 5M is *H-1*. Although there were apparent recombinants in line 21M, a number of these that were tested genetically proved to be false negatives, presumably the result of residual, weak histocompatibility differences in addition to the difference linked with *p*, and hence not crossovers. So far as the evidence goes, there may have been no recombination between *p* and the gene for resistance in this stock.

At this point the question arose whether the gene for resistance in line 21M was *H-1* or a representative of some new locus. This was tested by the F_1 test, with results summarized in Table 5.

TABLE 5

EVIDENCE FROM F_1 TYPING TEST CONCERNING THE LOCI WHICH DIFFERENTIATE IR LINES B10.BY, B10.129(5M), B10.129(21M) AND B10.129(20M) FROM C57BL/10*

Cross	Results		Conclusion re locus
	Susceptible	*Resistant*	
21M × 5M	21	0	21M is not *H-1*
5M × B10.BY	0	17	5M is *H-1*
20M × 21M	1†	10	20M is *H-4*
20M × 5M	10	0	20M is not *H-1*
5M × B10.LP	10	0	5M is not *H-3*
B10.BY × B10.D2	26	0	B10.BY is not *H-2*
B10.BY × B10.LP	41	1	B10.BY is not *H-3*
B10.BY × 21M	9	0	B10.BY is not *H-4*

* All mice immunized with C57BL/10 thymus, three times, and inoculated with C57BL/10 tumour S913. Some mice from the cross B10.BY × B10.D2 survived the first inoculation of the tumour, presumably due to the 'hybrid effect', but succumbed to a second inoculation.

† Late death.

The nature and significance of this test have been fully described elsewhere (Snell, 1958b). Briefly, the procedure is to cross two lines, in this instance 5M and 21M, and to challenge the F_1 with a tumour from a third strain co-isogenic with one or both of them, in this instance C57BL/10. We may then represent the two possible alternatives expected as follows:

	C57BL/10 (tumour donor)	F_1 hybrid 5M × 21M	Response of F_1 to transplant
One locus difference	$H\text{-}1^c/H\text{-}1^c$	$H\text{-}1^e/H\text{-}1^e$	Resistant
Two locus difference	$H\text{-}1^cH\text{-}4^a/H\text{-}1^cH\text{-}4^a$	$H\text{-}1^eH\text{-}4^a/H\text{-}1^cH\text{-}4^b$	Susceptible

If the hybrid is susceptible, it proves (in the case in which all three lines are on the same genetic background) that each of the two strains used as parents brings in the gene for susceptibility which the other lacks and, hence, differs from the tumour donor at *different loci*. The result in this case was twenty-one deaths in twenty-one mice. Hence, if 5M differs from C57BL/10 at *H-1*, 21M does *not* differ from C57BL/10 at *H-1*. We may therefore say that 21M identifies a new locus closely linked with *p* and may assign the locus the symbol *H-4*. The allele in C57BL/10 may be called $H\text{-}4^a$ and that in 21M, $H\text{-}4^b$.

A cross between B10.BY and 5M gave seventeen mice, all resistant. This confirms the conclusion that both lines are *H-1* lines. Other tests listed in the table show that 20M, which is both *c* and *p*, differs from C57BL/10 at *H-4* but not at *H-1*. Evidently the *H-1* allele of 129, which was introduced into 5M with *c*, was lost by crossing over between *H-1* and *c* in the production of 20M. Table 5 includes the results of several additional tests which all tend to confirm these conclusions.

In stocks C57BL/10, 5M, 20M and 21M there are, then, a total of four known first

linkage group gene differences, *H-1*, *c*, *p* and *H-4*. What is the order of these genes? The fact that in the production of line 20M, the *H-1* allele of 129 was lost, but the alleles *c*, *p*

TABLE 6

EVIDENCE CONCERNING ORDER OF LOCI *H-1*, *c*, *p* AND *H-4* FROM MATING ♀*H-1*^{c}++*H-4*^{a}/*H-1*^{e}*c*^{ch}*pH-4*^{b} × ♂*H-1*^{c}*cpH-4*^{b}/*H-1*^{c}*cpH-4*^{b}*

?++*H-4*^{a}	*H-1*^{c}+*pH-4*^{b}	*H-1*^{e}*c*^{ch}+*H-4*^{a}	?*c*^{ch}*pH-4*^{b}	*Total*
32	5*	6†	22‡	65

* All mice not manifesting a crossover between *c* and *p* were immunized and implanted with C57BL/10 tumour S913 (genetically *H-1*^{c}*H-4*^{a}). Of the thirty-two++ mice, twenty-two succumbed promptly, five succumbed after unusual delay, and five survived but were proved by progeny tests (see Table 7) to be *H-1*^{c}++*H-4*^{a} and hence genetically susceptible.

† Not inoculated with tumour; tested genetically for *H-1* and *H-4* allele (see Table 7).

‡ Survived inoculation of tumour S913. Crossover per cent between *c* and *p* = 16.9. No crossovers between *H-4* and *p*, and no proven crossovers between *H-1* and *c*, though these would have been detected only in the two classes involving a crossover in the c — *p* interval.

and *H-4*^{b} retained, establishes a presumption that *H-1* is not between *c* and *p*. It if were between these genes, a double crossover, an infrequent event in the mouse in this interval

TABLE 7

GENETIC TESTS OF BACKCROSS MICE LISTED IN TABLE 6

Genotype and mouse number	*Mated to*							
	5M (test for H-1 allele)				*21M (test for H-4 allele)*			
	Susceptible		*Resistant*		*Susceptible*		*Resistant*	
H-1^{c}++*H-4*^{a}	*C*	*c*	*C*	*c*	*P*	*p*	*P*	*p*
♀31	7	6	0	0	6	0	1	6
♂57	6	4	0	0	10	0	2	7
♂66	9	6	0	0	4	1	5	11
♀77	6	4	0	0	7	0	1	13
♀84	5	5	0	0	8	0	1	7
Totals	33	25	0	0	35	1	10	44
H-1^{c}+*pH-4*^{b}								
♀26	7	11	0	0		0		13
♀47	9	11	0	0		0		13
♂52	11	9	0	0		0		22
♂53	9	6	0	0		0		16
♂80	5	6	0	0		0		15
Totals	41	43	0	0		0		79
H-1^{e}*c*^{ch}+*H-4*^{a}	*c*^{ch}	*c*	*c*^{ch}	*c*				
♀36	2	2	5	1	4	1	7	11
♀61	1	6	8	2	8	0	1	6
♀62	0	4	8	0	9	0	0	10
♂72	0	7	5	0	7	0	1	4
♀73	0	5	7	0	13	0	0	7
♂87	1	8	5	0	5	0	5	10
Totals	4	32	38	3	46	1	14	48

(Grüneberg, 1952), would have been required. A linkage test was set up to obtain additional evidence.

The cross used was (B10.LP × 129) × B10.129(20M). The results, and additional details as to methods, are summarized in Tables 6 and 7. The first column of Table 6 includes five

mice that resisted the tumour but were proved by the genetic tests shown in Table 7 to be genetically susceptible. Also the matings to 21M shown in Table 7 reveal an obvious excess of resistant *P* mice. The 'hybrid effect' may have been a factor in producing these false negatives, but the fact that the cross was not segregating for any *H-2* difference and the conspicuous absence of false negatives from the test matings to line 5M argue against this. More probably their occurrence was due to a significant lack of co-isogenicity in lines 20M and 21M (the 21M mice used in test matings were from G14). The net result was to increase the number of test matings necessary, without seriously obscuring the final conclusions.

Out of sixty-five backcross mice, there were eleven crossovers between *c* and *p*. This gives a crossover rate of 17 per cent, in good agreement with established values (Grüneberg, 1952). In none of these eleven crossovers was there also a separation between *c* and *H-1*. If *H-1* lay between *c* and *p*, it is highly improbable that all eleven crossovers would have been between *H-1* and *p* and none between *c* and *H-1*. This, together with the evidence from the genotype of line 20M, makes it virtually certain that the order of these genes is *H-1cp*. While there are several animals listed in Table 7 that may represent crossovers between *p* and *H-4*, none of these was tested genetically. None of eleven crossovers of Table 6 involved an exchange between *p* and *H-4*. All we can conclude is that the linkage between *p* and *H-4* is close, with the order undetermined. Conceivably *H-4* is identical with the *p* locus itself.

The last group of test mice in Table 7 from the mating to 5M was genetically a backcross with segregation for *H-1* and *c*. These animals provide useful linkage information. The data may be combined with previously published backcross data (Snell, 1958b) The combined results show 8.5 ± 2.1 per cent crossing over in the female and 5.4 ± 3.7 per cent crossing over in the male. These may be taken as the best available values for the *H-1* — *c* interval.

The *H-1* alleles in C3H and C3H.K have been assigned the symbols $H\text{-}1^a$ and $H\text{-}1^b$ respectively (Snell, 1958b). Evidence as to the *H-1* alleles in C57BL/10, in the various sublines of C57BL/10 described in this paper and in certain standard inbred strains, was sought by the F_1 test, with the results shown in Table 8. In these tests one of the three strains (the 'unknown') was non-co-isogenic with the other two, and the tests were used to gain information about alleles and not about loci. (Compare Table 5.) As employed in this context, significance of the test may be illustrated by two of the cases shown in Table 8 in which strains C3H and C3H.K constituted the co-isogenic 'test pair', and C57BL/10 (case 1 in the table) and DBA/1 (case 10) the 'unknown'. In both tests, C3H.K was crossed to the 'unknown', and the resulting F_1 hybrids triply immunized and challenged with C3H tumour E9514. Where C57BL/10 was the unknown, all of ten offspring were resistant; where DBA/1 was the unknown, all of ten offspring were susceptible. In the latter case, DBA/1 contributed the element for susceptibility which C3H.K lacked, in other words it was proved to be $H\text{-}1^a$. C57BL/10 did not contribute the missing element; it was found to be not $H\text{-}1^a$. The test with DBA/1 has been repeated a number of times, always with the same result.

In the early applications of the F_1 test, mice were singly rather than triply immunized. Usually there were deaths in all test groups, but in some of them, as when DBA/1 or DBA/2 was the unknown, all mice died and the deaths were prompt, whereas in other groups only a fraction of the mice died and the deaths were delayed. Two tests of the latter type are included in the table; strains 129 and C57L were the unknowns. The results are

regarded as negative and the lines classed as not *H-1*^{a}. With triple immunization and the test systems employing tumours E9514 and S913, remarkably clear and consistent results were obtained.

The results in Table 8 establish C57BL/10 as the bearer of a new *H-1* allele, *H-1*^{c}, and B10.BY and B10.129(5M) as being not *H-1*^{a}. (Of necessity, these lines are also not *H-1*^{c} if, as indicated earlier in this paper, they differ from C57BL/10 with respect to *H-1*.) Attempts to get additional information about the alleles in these strains by the F_1 test, using tumours indigenous to strains B10.BY and C3H.K, gave unsatisfactory results because these particular tumours failed almost entirely to discriminate between susceptible and resistant animals differing at *H-1*. It has been noted previously (Snell, Smith and Gabrielson, 1953; Snell, 1957) that tumours are not uniformly suitable for genetic studies. Recourse was therefore had to the 'cross immunization test' (Snell, Wheeler and Aaron, 1957), with the results shown in Table 9.

TABLE 8

EVIDENCE FROM F_2 TYPING TEST CONCERNING THE *H-1* ALLELE IN VARIOUS IR AND INBRED STRAINS

Tumour donor	*Cross*	*Immunization*	*Results*		*Conclusions re allele*
			Susceptible	*Resistant*	
C3H	C3H.K × C57BL/10	3×	0	10	C57BL/10 is not *H-1*^{a}
C57BL/10	B10.BY × C3H	3×	0	10	,,
,,	B10.BY × C3H.K	3×	0	10	C57BL/10 is not *H-1*^{b} Is *H-1*^{c}
C3H	C3H.K × B10.BY	3×	0	10	B10.BY is not *H-1*^{a}
,,	C3H.K × B10.129(5M)	3×	0	10	B10.129(5M) is not *H-1*^{a}
,,	C3H.K × 129	1×	7	5	129 is not *H-1*^{a}
C57BL/10	B10.BY × 129	3×	0	10	129 is not *H-1*^{c}
,,	B10.BY × DBA/1	3×	0	10	DBA/1 is not *H-1*^{c}
,,	B10.BY × DBA/2	3×	0	8	DBA/2 is not *H-1*^{c}
C3H	C3H.K × DBA/1	3×	10	0	DBA/1 is *H-1*^{a}
,,	C3H.K × DBA/2	3×	19	0	DBA/2 is *H-1*^{a}
,,	C3H.K × C58	3×	0	10	C58 is not *H-1*^{a}
,,	C3H.K × C57L	1× *	10	7	C57L is not *H-1*^{a}
C57BL/10	B10.BY × C58	3×	10	0	C58 is *H-1*^{c}
,,	B10.BY × C57L	3×	8	0	C57L is *H-1*^{c}

* Immunized with 4 million C3H embryo cells.

Tumour C1498 rather than S913 was used in this case, since the test requires a high (preferably 100 per cent) mortality in homografted, unimmunized recipients. That C1498 is native to subline 6 rather than 10 of strain C57BL is a disadvantage but probably did not materially alter the results. The transplants grew in all unimmunized B10.BY and B10.129(5M) hosts, giving a consistent mean survival time (in groups of ten mice) of 20 to 22 days. In groups of mice of either strain triply immunized with C57BL thymus there were some survivors, and the survival of the mice that succumbed was usually prolonged. In groups of B10.BY mice immunized with 5M and C3H.K thymus there was also demonstrable, though less pronounced, protection. Lines 5M and C3H.K could not immunize line B10.BY against a tumour differing from B10.BY only (or almost only) at *H-1* unless they *also* differed from B10.BY at *H-1*. Hence we may infer that the *H-1* allele in B10.BY is not *H-1*^{b} since this is the allele in C3H.K. Since B10.BY is not *H-1*^{a}, *H-1*^{b} or *H-1*^{c}, we may assign its allele the symbol *H-1*^{d}. Likewise the allele in 5M is indicated as not *H-1*^{d}. An additional indication of non-identity between B10.BY and B10.129(5M) is provided

by evidence that the genetic gap between C57BL/10 and B10.BY is greater than that between C57BL/10 and B10.129(5M). In tests shown in both Table 2 and Table 9, B10.BY showed the greater resistance to C57BL tumours. The allele in B10.129(5M) is

TABLE 9

TEST FOR DIFFERENCES IN *H-1* ALLELES IN CO-ISOGENIC STRAINS ON A C57BL/10 BACKGROUND BY CROSS IMMUNIZATION, WITH CHALLENGE BY C57BL/6 TUMOUR C1498

Host	*Thymus donor*	*Results*		*Comments and conclusions*
		Percentage dying (10 mice)	*Mean survival (days)*	
B10.BY	None	100	20.1	Non-immune control
,,	B10.BY	100	21.9	Non-immune control
5M	5M	100	21.1	Non-immune control
B10.BY	C57BL/10	20	72.0	Immune control
5M	C57BL/10	80	48.7	Immune control. The weaker resistance shown by 5M is evidence that the allele in this strain is different from the one in B10.BY.
B10.BY	5M	100	23.7	One mouse survived 51 days. Such survival is never observed in controls. Induction of resistance and hence a difference between 5M and B10.BY is suggested.
5M	B10.BY	100	20.0	No evidence of difference. Note that 'weaker' strain is used as host in this test.
B10.BY	C3H.K	90	26.8	Resistance induced; *H-1* allele in C3H.K probably differs from that in B10.BY.
5M	C3H.K	100	22.0	No evidence of difference.

proved to be neither $H\text{-}1^a$, $H\text{-}1^c$ nor $H\text{-}1^d$, but evidence as to the identity or non-identity with $H\text{-}1^b$ is lacking.

Table 10 summarizes the available information concerning the number and distribu-

TABLE 10

DISTRIBUTION OF *H-1* ALLELES IN VARIOUS CO-ISOGENIC AND INBRED STRAINS

Allele	*Typed strains*
$H\text{-}1^b$	C3H DBA/1 DBA/2
$H\text{-}1^a$	C3H.K
$H\text{-}1^c$	C57BL/10 C57L C58
$H\text{-}1^d$	B10.BY
Not $H\text{-}1^a$, $H\text{-}1^c$ or $H\text{-}1^d$	B10.129(5M)
Not $H\text{-}1^a$ or $H\text{-}1^c$	A AKR BALB/c LP ST SWR 129*

* Data for this group of strains are not included in this paper. The data are essentially the same as other data shown in Table 8.

tion of *H-1* alleles. It is noteworthy that in both cases where one allele is shared by more than one strain ($H\text{-}1^a$ in C3H, DBA/1 and DBA/2, and $H\text{-}1^c$ in C57BL/10, C57L and C58),

the strains have known common ancestry (Snell, Staats, Lyon, Dunn, Grüneberg, Hertwig and Heston, 1960).

Table 11 shows the results of homografts of ovary and skin between co-isogenic strain pairs with *H-1* differences. A control group with *H-1* plus *H-2* differences also received ovary grafts. If the skin grafts are considered first, it is evident that there was relatively long survival, that there was considerable individual variability and that means in reciprocal directions were different. Skin of C3H.K transplanted to C3H survived an average of 36 days, whereas the mean survival in the opposite direction was 91 days. In the C57BL/10 — B10.129(5M) pair the survival times were 32 and 50 days. The grafts showed recurrent crises which sometimes made the end point difficult to determine. There is a suggestion of greater variability of survival time with decreasing 'strength' of the antigenic difference. All these phenomena have been observed previously in grafts between strains with *H-3* differences (Counce, Smith, Barth and Snell, 1956; Berrian and McKhann, 1960). Previous tests of skin grafting from C3H to C3H.K (Counce, *et al.*, 1956; Winn, Stevens and Snell, 1958) have shown long survival. In one study the ultimate outcome was rejection, while in the other, a test accompanied by tissue injections that may have modified the outcome, there was survival to the termination of the test at intervals ranging from 60 to 170 days. The present test makes it clear that there is a potential resistance to skin grafts in this donor-host combination, though very weak. One graft survived 124 days.

Ovarian grafts from C3H.K to C3H.SW, a donor-host combination with both an *H-1* and an *H-2* difference, produced no young. The *H-2* difference, as expected, caused effective graft rejection. In a compatible combination, C3H.K to (C3H × C3H.K), eight out of nineteen grafted females produced young, with an average of 10.7 young per producer, and the last parturition occurring at intervals from 44 to 180 days after the graft. The *H-1* combination, C3H.K to C3H, gave fully equivalent results, with six of ten grafts producing 12.5 young per producer, and the last parturition at 75 to 215 days. Even with previous immunization, four of eighteen grafts survived 132 to 160 days. Since skin grafts in this combination were rejected in 29 to 54 days without immunization, and in 16 to 43 days with immunization, it is clear that ovarian grafts, at least in this particular genetic context, have the greater capacity to survive (and/or possibly a lower capacity to incite) the homograft reaction.

DISCUSSION

The frequency and ease with which *H-1* differences have been detected in studies based on tumour transplantation and the relative 'weakness' of this locus with respect to skin grafts are noteworthy. The existence of two additional *H-1* lines not sufficiently analysed to include in this report serves to further emphasize this point. We may speculate that this indicates a relatively high concentration of the *H-1* antigen in lymphoid tissue as compared with skin.

The results here reported also have interesting implications as to the number of histocompatibility loci. Barnes and Krohn (1957), using skin grafts to F_2 mice, arrived at an estimate of not less than fifteen loci. Subsequently Hicken and Krohn (1960) carried out a similar experiment using ovarian grafts, and obtained a ratio indicating about eleven loci. Because mice which tolerated the ovarian grafts subsequently tolerated skin grafts, these authors attached considerable weight to the second figure. They further favoured the later figure because they found no evidence 'that ovarian grafts, whether orthotopic

TABLE 11

SURVIVAL OF HOMOGRAFTS OF OVARY AND SKIN BETWEEN CO-ISOGENIC STRAIN PAIRS WITH *H-1* DIFFERENCES

Histocompatibility difference	*Donor*	*Host*	*Ovarian grafts*				*Skin grafts*		
			Number producing/number grafts	*Number young per producer*	*Age graft at last parturition*	*Stimulated uteri**	*Number*	*Survival (days)*	
								Mean	*Range*
H-1 and *H-2*	C3H.K	C3H.SW	0/10	—	—	4/10			
None	C3H.K	(C3H × C3H.K)†	8/19	10.7	44–180	19/19			
H-1	C3H.K	C3H	6/10	12.5	75–215	9/9	7	36	29–54
H-1	C3H.K	C3H†	4/18	5.8	132–160	17/17	5	29	16–43
H-1	C3H	C3H.K					6	91	35–124
H-1	C57BL/10	B10.129(5M)	2/9	3.5	108–121	9/9	4	32	25–39
H-1	B10.129(5M)	C57BL/10					6	50	20–95

* When autopsied at about 200 days post-operation. Ovariectomized females of strain C3H sometimes show uterine stimulation due to adrenal secretions; C57BL/10's do not.

† Immunized with one intraperitoneal injection of C3H.K thymus, 40 million cells per mouse, 11 days prior to grafting.

or subcutaneous, exchanged between two strains of mice are less susceptible to the homograft reaction than skin grafts'.

Examination of their methods shows that this conclusion was based on grafts involving either *H-2* differences or multiple non-*H-2* differences. It is perfectly clear from our results that, when strains differ at the *H-1* locus only, ovarian grafts survive much longer than skin grafts. We have no evidence as to other loci, but it seems likely to us that this difference is more nearly a characteristic of weak loci in general than of the *H-1* locus in particular. If so, the different gene estimates from skin grafts and ovarian grafts are partly explained. A clue as to the meaning of the successful outcome of skin grafts, used to check the ovarian successes, comes directly from the same study. The experiments 'provided a considerable number of instances (particularly with CBA donor material) where a second ovarian graft has survived for longer, and often for much longer, than the first graft. These rather unexpected results bear at least some superficial resemblance to the phenomenon of enhancement and might be explained in similar terms'. Whether this is enhancement or tolerance or yet a different condition is immaterial for the present argument; the important point is that when the genetic gap is narrow enough a graft seems to induce some sort of tolerant state even in the adult. This is a finding with obviously important implications. Its significance here is that the arguments for accepting the 11 gene rather than the 15 gene estimate are removed.

As Barnes and Krohn were quite aware, their estimate of fifteen loci was a minimum one. Our results highlight two factors that may push up such an estimate. The first is linkage; *H-1* and *H-4*, with about 24 per cent crossing over between them, would contribute less to the gene estimate derived from a segregating generation than would unlinked factors. The second is the existence of very weak loci. Some of our C3H to C3H.K skin grafts survived for more than 100 days, and hence would be counted as successes by Barnes and Krohn. The C57BL/10 — B10.129(5M) gap is either greater, owing to the different *H-1* alleles involved, or the C57BL background is less favourable than C3H to prolonged survival, but even in this case one 5M graft survived 95 days. The *H-1* gap, in some allelic combinations at least, is so weak that it would sometimes escape detection in the case of individual normal tissue grafts, but data in Table 2 suggest that the *H-4* locus is even weaker. When mice were unimmunized, there were one out of twenty-three (B10.BY hosts) or eight out of twenty-eight (5M hosts) deaths where there was an *H-1* difference, and nine out of ten (21M hosts) where there was an *H-4* difference. The 'contaminant' locus (or loci) still segregating in line 21M at G14 was apparently weaker still. Whereas one or two immunizations produced good survival of *H-4* resistant mice, it took three immunizations to produce more than a very small number of survivors due to the 'contaminant'. The histocompatibility difference or differences between C57BL/6 and C57BL/10, presumably derived by mutation, are also in the ultra weak category (Table 2). One word of caution is necessary: we cannot infer from a single instance of a very weak histocompatibility difference that the *locus* concerned is weak; it may be merely that a particular pair of alleles at the locus is very much alike, whereas other pairs would present a wider histocompatibility gap. Nevertheless the evidence should serve to remind us that there may be many very weak loci which escape detection in most studies.

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